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PROJECT UPDATE

A green chemical approach for the synthesis of gold nanoparticles: characterization and mechanistic aspect

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Abstract This IRCSET-EMPOWER (Irish Research Council for Science, Engineering and Technology Postdoctoral Research Grant) project aims to improve current methodology for the synthesis of metal nanoparticles (NPs). The development of efficient methodology for metal nanomaterials synthesis is an economical and environmental challenge. While the current methods for NPs synthesis are often energy-intensive and involve toxic chemicals, NPs biosynthesis can be carried on at circumneutral pH and mild temperature, resulting in low cost and environmental impact. Nanomaterial biosynthesis has been already observed in magnetotactic bacteria, diatoms, and S-layer bacteria, however, controlled NPs biosynthesis is a relatively new area of research with considerable potential for development. A thorough understanding of the biochemical mechanism involved in NPs biosynthesis is needed, before biosynthetic methods can be economically competitive. The analysis and identification of active species in the nucleation and growth of metal NPs is a daunting task, due to the complexity of the microbial system. This project work focuses on the controlled biosynthesis of gold NPs by fungal microorganisms and aims to determine the biochemical mechanism involved in nucleation and growth of the particles.

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1 Introduction

The synthesis of metal nanoparticles (NPs) is a growing area of research in materials science because they exhibit unique properties (Gratzel 2001; Xia et al. 2003), different from those of bulk metals due to their unique size and shape dependent characteristics. Because of stability, oxidation resistance, and biocompatibility, gold NPs find wide applications in electronics and photonics, catalysis, information storage, chemical sensing and imaging, drug delivery and biological labeling (Elghanian et al. 1997; Cao et al. 2002). For each application, NPs of different size and shape are needed (Jin et al. 2001; Sun and Xia 2002), thereby synthetic protocols for the production of size and shape controlled monodisperse NPs are required. Since Faraday's pioneering work in 1857 on the synthesis of colloidal gold by reducing $NaAuCl_4$ with a solution of phosphorus in carbon disulphide (Fig. 1) (The Royal Institution of Great Britain 2008; Thomson 2007), several physical and chemical methods have been developed to produce gold NPs. Synthetic techniques based on the reduction of metal ions with sodium citrate or sodium borohydride, followed by surface modification of the

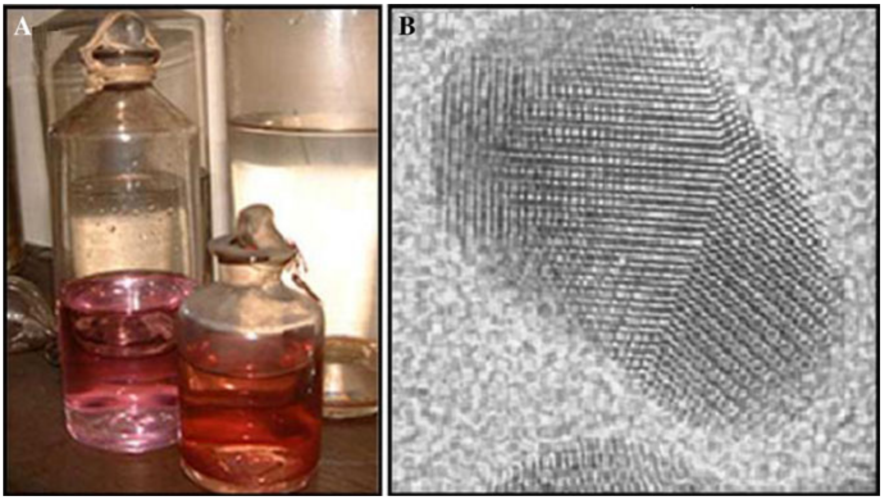

Fig. 1 Faraday's colloidal suspension of gold (**a**) (The Royal Institution of Great Britain 2008); High resolution transmission electron microscopic image (**b**) of individual colloidal gold particles (at a magnification of 10^7 ×), prepared according to Faraday's recipe (Edwards, Thomas 2007)

produced particles with suitable capping ligands and organic solvents (Niemeyer et al. 1998; Lévy et al. 2004), raised environmental concerns, because of the toxic compounds used in the process. Also, it is difficult to obtain NPs of defined size and shapes (e.g., spheres, rod, cubes, hexagons, etc.) in high yield. Current synthetic methods result in mixed-shape NPs that require expensive and low-yield purification procedures, such as differential centrifugation (Murphy 2002). These limitations invite new eco-friendly ("green chemistry") methodology for production of nanocrystals with desired shape.

Natural processes such as biomineralization may be mimicked to design efficient NP synthesis techniques. Biomineralization processes exploit biomolecular templates that interact with the inorganic material throughout its formation resulting in the synthesis of particles with defined shape and size (Mann 1993). Bones, teeth and shells are typical examples of structural materials produced by natural biomineralization processes. A general scheme for NP synthesis in microorganisms is illustrated in Fig. 2.

The adoption of biomineralization methods in the synthesis of nanostructured materials is expected to yield novel and more complex structural entities compared to those obtained with conventional methods (Brown et al. 2000; Klaus et al. 1999; Mukherjee et al. 2001; Xie et al. 2007a, 2007b). Both uni and multicellular microorganisms are reported to produce inorganic nanomaterials either intra- or extracellularly. Some well-known examples (Mann et al. 1990; Oliver et al. 1995; Sleytr et al. 1999) of microbial mediated synthesis of inorganic materials include magnetotactic bacteria *Magnetospirillum magnetotacticum* (which synthesize magnetite NPs), S-layer bacteria *Synechococcus* sp., *Bacillus stearothermophilus* (which produce gypsum and calcium carbonate layers), and diatoms *Coscinodiscus* sp., *Cylindrotheca fusiformis* (which synthesize siliceous materials). However, the use of microorganisms in the deliberate and controlled NP synthesis is a new area of research.

1.1 Bacteria in nanoparticle synthesis

Among the microorganisms, prokaryotes have received the most attention in the area of NP biosynthesis. Beveridge and Murray (1980) have demonstrated that gold NPs readily precipitate in bacterial cells following incubation of the cells with Au^{+3} ions under ambient temperature and pressure. Organic phosphate compounds play a role in the in vitro development of octahedral Au, possibly as bacteria–Au complexing agents. Fe^{+3} reducing bacteria *Shewanella algae* can reduce Au^{+3} ions in anaerobic environments. In the presence of *S. algae* and hydrogen gas, the Au ions are completely reduced and 10–20 nm gold NPs are formed (Konishi et al. 2004). Klaus–Joerger and co-workers (Klaus et al. 1999) have demonstrated that *Pseudomonas stutzeri* AG259 isolated from a silver mine reduces Ag^{+} ions and forms silver NPs of well-defined size and morphology, ranging from a few to 200 nm or more, within the periplasmic space.

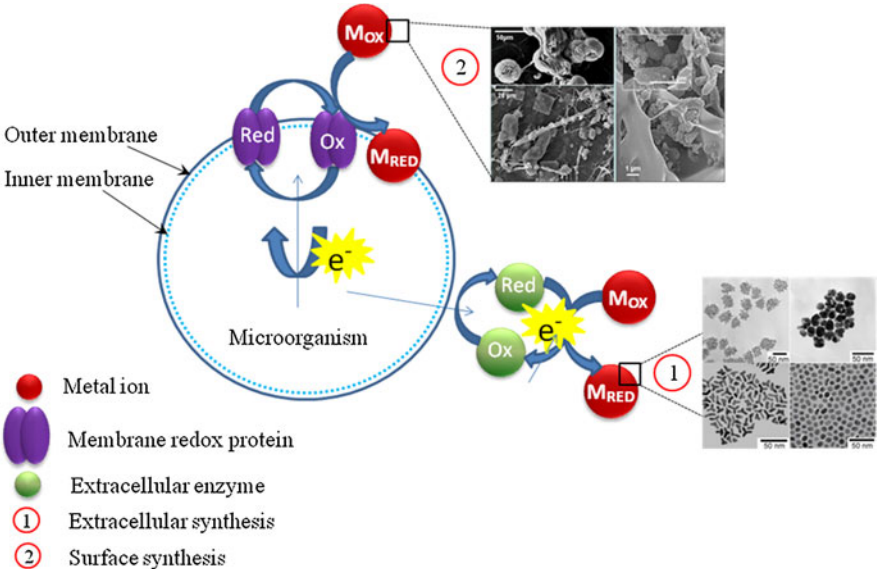

Fig. 2 Schematic of biomineralization process for nanoparticle synthesis

1.2 Fungi in nanoparticle synthesis

The use of fungi in the synthesis of NPs is a relatively recent addition and holds promise for large scale NP production. In fact, fungi secrete large amounts of the enzymes involved in NP synthesis and are simpler to grow both in the laboratory and at industrial scale. Different fungal and actinomycete species, i.e., *Fusarium oxysporum*, *Verticillium* sp., *Thermomonospora* sp., *Rhodococcus* sp. have been reported (Ahmad et al. 2003; Mandal et al. 2006) to synthesize NPs intra- or extracellularly. Shankar et al. (2004) synthesized gold nanoplates by fungal extracts.

A brief over view on microbial synthesis of metal NPs is given in Table 1.

1.3 Mechanism of nanoparticle synthesis

While a large number of microbial species are capable of producing metal NPs, the mechanism of nanoparticle biosynthesis has not been established. The metabolic complexity of viable microorganisms complicates the analysis and identification of active species in the nucleation and growth of metal NPs. Recent works by Xie et al. (2007a) demonstrated that proteins are the principal biomolecules involved in the algal

Table 1 Synthesis of nanoparticles by different microorganisms

Microorganisms	Metal nanoparticle	References
Bacteria		
Bacillus subtilis	Gold	Beveridge and Murray (1980)
Shewanella algae	Gold	Konishi et al. (2004)
Pseudomonas stutzeri	Silver	Klaus et al. (1999)
Lactobacillus	Gold, silver, Au–Ag alloy	Nair and Pradeep (2002)
Escherichia coli	Gold	Brown et al. (2000)
Rhodococcus	Gold	He et al. (2007)
Fungi		
Verticillium	Gold, silver	Mukherjee et al. (2001)
Fusarium oxysporum	Gold, silver, Au–Ag alloy	Ahmad et al. (2003); Mandal et al. (2006); and there in
Colletotrichum sp.	Gold	Shankar et al. (2003)

synthesis of gold NPs. Other researchers (Ahmad et al. 2003; He et al. 2007) have postulated that microorganisms secrete enzymes, which may be responsible for the reduction of metal ions, which result in the NPs nucleation and growth. Ahmad et al. (2003) postulated that a NADH-dependent reductase is involved in Ag NPs synthesis by *Fusarium oxysporum*. However, the biochemical mechanism of metal ion reduction and subsequent NP formation remains unexplored.

The elucidation of the biochemical pathways leading to gold biomineralization is necessary to develop a rational approach to NP biosynthesis. A number of issues need to be addressed from the nanotechnology and microbiological points of view before such biosynthetic procedures can compete with the conventional protocols.

Preliminary experiments (Fig. 3) carried out in our laboratory demonstrated the synthesis of single crystal gold NPs from $HAuCl_4$ when incubated with mycelia or cell-free extract of *Rhizopus oryzae* (Das et al. 2008, 2009).

‘Green chemistry’ aims to employ environmentally benign solvents and nontoxic chemicals in synthetic methods, thereby reducing their environmental impact (Anastas and Warner 1998). This IRCSET-EMPOWER project will adopt such ‘green chemistry’ approach to synthesize gold NPs using microorganisms as a ‘living nano-factory’ by avoiding any chemical agents, to determine the biochemical mechanisms involved in the biomineralization process, and to isolate and purify the enzymes involved in the NP formation.

2 Methodological approaches

The present project work aims to (1) synthesize gold NPs through room temperature reduction of gold ions by *R. oryzae*; and (2) determine of the biochemical mechanism involved in the nucleation and growth of gold NPs. *R. oryzae* will be grown in the laboratory as previously described (Das et al. 2009) and the cell-free

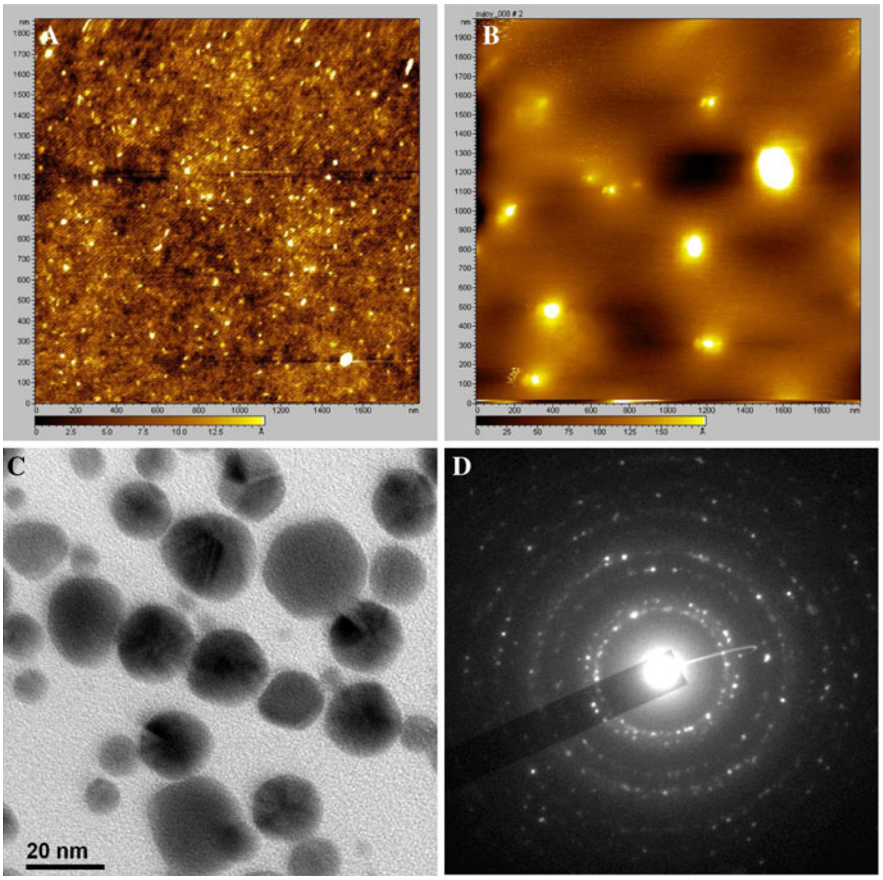

Fig. 3 Atomic force microscopic images (**a**–**b**) of gold nanoparticles synthesized on the surface of the fungal mycelia (**a** low resolution; **b** high resolution); high resolution transmission electron microscopic image (**c**) shows that the average particles size is 20 nm; selected area electron diffraction (SAED) pattern (**d**) indicates that the synthesized gold nanoparticles are single crystal

extract will be prepared from *R. oryzae* mycelia following harvesting from the growth medium. The cell-free extract will then be used for the synthesis of gold NPs.

The NP will be characterized through Transmission Electron Microscopy (TEM), Atomic Force Microscopy (AFM), X-ray photoelectron spectroscopy (XPS), UV–visible and Fourier Transform Infrared spectroscopy (FTIR). The crystal structure of the NPs will be determined with the selected area electron diffraction (SAED) pattern obtained from TEM image.

The biochemical pathway involved in the NP biosynthesis will be assessed using heat inactivated or metabolically inhibited microorganisms (e.g., through sodium cyanide, formaldehyde, and 2,4-dinitrophenol) as control experiment. The enzyme(s) responsible for reduction of Au^{+3} to Au^0 will be obtained from the cell-free extract through several purification steps: ammonium sulphate fractionation, anion-exchange chromatography, chromatofocusing, and gel filtration. The purified enzyme(s) will then be used for the synthesis of gold NPs as described above. Kinetic measurements of enzyme activity for gold NP synthesis will then be undertaken at the optimal pH and temperature, as determine in separate experiments. The shape controlled synthesis of gold NPs by purified enzyme(s) will be also performed using varying reaction conditions, such as the concentration of gold ions, the pH of the solution, and the incubation period. All these conditions control the crystal growth kinetics and final NP morphology. Since the enzyme(s) secreted by *R. oryzae* act both as reducing and capping agent, the adsorption of such enzyme(s) on the growing crystals and their reducing activity changes with the above mentioned conditions, thereby resulting in different crystal shapes.

3 Impact of the proposed research

Metal NPs are relevant to numerous emerging technologies. The development of high yield and low cost methods for NP production is therefore an important challenge. Current methods for metal NP production require harsh chemicals and energy-intensive processes. It is consequently important to develop an eco-friendly sustainable (“green chemistry”) alternative to the existing chemical methods. The microbial-mediated synthesis of metal NPs may replace some of the current physical and chemical methods in use for NP production. However, several issues need to be addressed before such biosynthetic procedures can compete with established protocols. Comprehension of biochemical mechanism involved in gold nanoparticle formation is crucial to the development of innovative and low-energy NPs production processes. This IRC-SET-EMPOWER project deals with pioneer research on the synthesis of gold nanostructures employing microorganism simultaneously as the reducing and capping agent. This environmental-friendly methodology may be applied in various pharmaceutical and biomedical formulations, as well as in cellular imaging, biosensing, and drug delivery.

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